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Role of Toll-Like Receptor (TLR)-Signaling in Cancer Progression and Treatment

Shyam Babu Prasad and Rahul Kumar

Abstract

Toll-like receptors (TLRs) are the most essential pattern recognition receptors in mediating the effects of innate immunity. It plays a pivotal role in inducing immune response against a number of pathogens, various diseases conditions including pathogenesis of cancer. Inflammation is often associated with the development and progression of most of cancer, where TLRs interplay very crucial roles. Moreover, TLRs activation can impact the initiation, progression and treatment of cancer by modulating the inflammatory microenvironment. Rapidly growing number of evidences related to TLRs function and expression in cancer cells, suggests its critical association with chemoresistance and tumourigenesis. The current chapter describes the development of various agonist and antagosit for TLRs and their application in cancer therapeutics. The aim of this book chapter is to highlights basic features of TLRs, and its role in cancer progression. It also addresses, how a defect in the TLRs signaling pathway can contributes towards carcinogenesis and recent development of cancer therapeutics that target TLR signaling pathways.

Keywords: toll-like receptors (TLRs), cancer progression, TLR agonists, inflammation, signaling

1. Introduction to the toll-like receptors (TLRs)

TLRs are trans-membrane proteins receptors that trigger the signal transduction cascades upon binding with specific pathogen-associated molecular patterns (PAMPs) ligands, and earlier have been thought to be restricted to immune cells. TLRs play a key role in the innate immune system as well as subsequent induction of adaptive immune responses against microbial infection or tissue injury [1]. TLRs receptors triggers immune response against various invading pathogens by recognizing receptor specific to PAMPs, which is highly conserved and derived from potential pathogenic microorganism such as bacteria, viruses, fungi and parasites [2, 3]. The very well-known one such PAMPs is lipopolysaccharides (LPS) acts as ligands for TLRs, which is found on outer cell wall of gram negative bacteria [4]. Moreover, TLR receptors also recognize endogenous damage-associated molecular patterns (DAMPs), derived from injured host cells including necrotic cancer cells, dead or dying cells, or products released from cells in response to signals such as hypoxia and epithelial cells [5]. These PAMPs and DAMPs together help

in discriminating both self and non-self-danger signals [1, 2]. Specific TLR receptors recognize distinct microbial ligands i.e. lipopeptides, lipoteichoic acids, LPS, peptidoglycans, flagellins, viral and bacterial nucleic acids etc. [6]. These ligands bind to specific TLR receptors, initiate cascade pathway which plays an important role in maintenance of cellular homeostasis, cell proliferation or apoptosis, cell differentiation, as well as induction of inflammatory cytokines like interferons (IFNs), interleukins (IL2, IL6, IL8, IL12, IL16), and TNF- α to get rid of pathogens [3, 7].

Cancer develops when uncontrolled growth of abnormal cells occurs anywhere in a body and further metastasized to distant part of the body. In order to deepen our understanding of cancer biology, it is very important to address the factors that are involved in the tissue repair process, such as cytokines, chemokines, growth factors and TLR signaling, which are the key determinants of cancer progression [8, 9]. TLR signaling is known to activate nuclear factor- κ B (NF κ B) and mitogen-activated protein kinase (MAPK) pathways [10]. NF- κ B in turn, regulates the expression of anti-apoptotic genes, and activation of the complement pathway depending upon type of ligands it sensed [11, 12]. Furthermore, TLRs are expressed not only on the surface of immune cells but also on cancerous cells [13]. In humans, TLRs (TLR1-TLR10) play a very important role in disease progression and the TLRs signaling have been well studied in various diseases including cancer [14]. The TLRs and their intracellular signaling components play a very important role in the onset of inflammatory diseases [4]. Recent studies have revealed that chronic inflammation can increase the risk of cancer development and also promote its progression [14]. TLRs signaling also plays a crucial role in the development of chemo-resistance; *Michael et al.*, (2006) shows TLR4/MYD88 signaling promotes tumor growth and contributes to chemo-resistance against paclitaxel in ovarian cancer [9]. Moreover, a recent study delineates that high TLR7 and TLR8 expression promotes chemo-resistance, leading to increased tumor cell proliferation in human pancreatic cancer [15]. However, the role of TLR signaling is still not completely understood in cancer progression; some studies suggest it has both pro-tumor as well as anti-tumor effects. To date, TLRs are documented to play a supportive role for initiation, progression and metastatic potential of cancer [16, 17]. On the other hand, they are capable of maintaining an antitumor environment by eliciting activation of anti-tumor mediators such as type I interferon [18]. This book chapter highlights the current understanding of role of TLRs and addresses a crucial link between carcinogenesis and immune cells, TLRs signaling and antagonist.

2. TLRs genetics and regulation of signaling

TLRs were first described in *Drosophila* in 1984, and were later discovered in vertebrates including humans [1, 3]. Till date, 13 TLRs are discovered in mammals, and 10 are functional in humans [2]. Genes encoding human TLRs are located on chromosomes 1 (TLR5), 3 (TLR9), 4 (TLR1, TLR2, TLR3, TLR6 and TLR10), 9 (TLR4) and X (TLR7 and TLR8) [1, 5]. TLR1–9 is conserved in both human and mice; however, mouse TLR10 is not functional because of a retrovirus insertion, and TLR11–13 has been lost from the human genome [8, 19, 20].

A number of genetic changes like single nucleotide polymorphisms (SNPs) within the TLR genes has been reported in humans which can influence receptor binding and function, that ultimately influences the risk for the inflammatory diseases as well as cancers [21]. Although there have been numerous studies reporting the impact of polymorphisms on TLR function and disease development, there is still a lot of contradiction in terms of outcomes [22].

A recent report has shown that functional TLRs are expressed not only on immune cells, but also on cancer cells, thus implicating a role of TLRs in cancer biology. Overwhelming evidence supports that TLR signaling provides a micro-environment that is necessary for tumor cells to proliferate and evade the immune response for further growth and migration [23]. The TLR family can be largely divided into 2 subgroups, extracellular and intracellular, depending on their cellular localization. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are located on the cell surface, while TLR3, TLR7, TLR8, and TLR9 are localized to the endosomal/ lysosomal compartment [10]. The subcellular localization of TLR4 is unique because it is localized to both plasma membrane as well as endosomal vesicles [24]. TLRs are type I transmembrane proteins that consist of three major domains: (1) a leucine rich extracellular domain, (2) a transmembrane domain, (3) A cytoplasmic TIR (Toll/Interleukin-1 Receptor) domain. The recognition of ligand by TLRs is mediated by the extracellular domain that harbor a leucine rich repeat (LRR) composed of 19–25 tandem copies of the “xLxxLxLxx” motif [25]. TLR signaling was extensively studied in the recent years. There are two important TLR pathways: one is dependent on myeloid differentiation factor 88 (MYD88) adaptor proteins and the other is independent of MYD88.

All TLRs except TLR3, which exclusively uses the TIR-domain-containing adapter-inducing interferon- β (TRIF) pathway, use MYD88 as the downstream adapter protein that activate the classical/canonical inflammatory signaling pathway [26–29]. After activation with their specific ligands, TLRs recruit MYD88, leading to subsequent activation of three main transcription factors: interferon-regulatory factors (IRF3, IRF5 and IRF7), NF- κ B, MAPK and AP1 [21–25, 27–32]. Subsequently, it promote the transcription of cytokines such as TNF- α , IL-6 and IL-1, chemokines and interferons which are key mediators of inflammation [30].

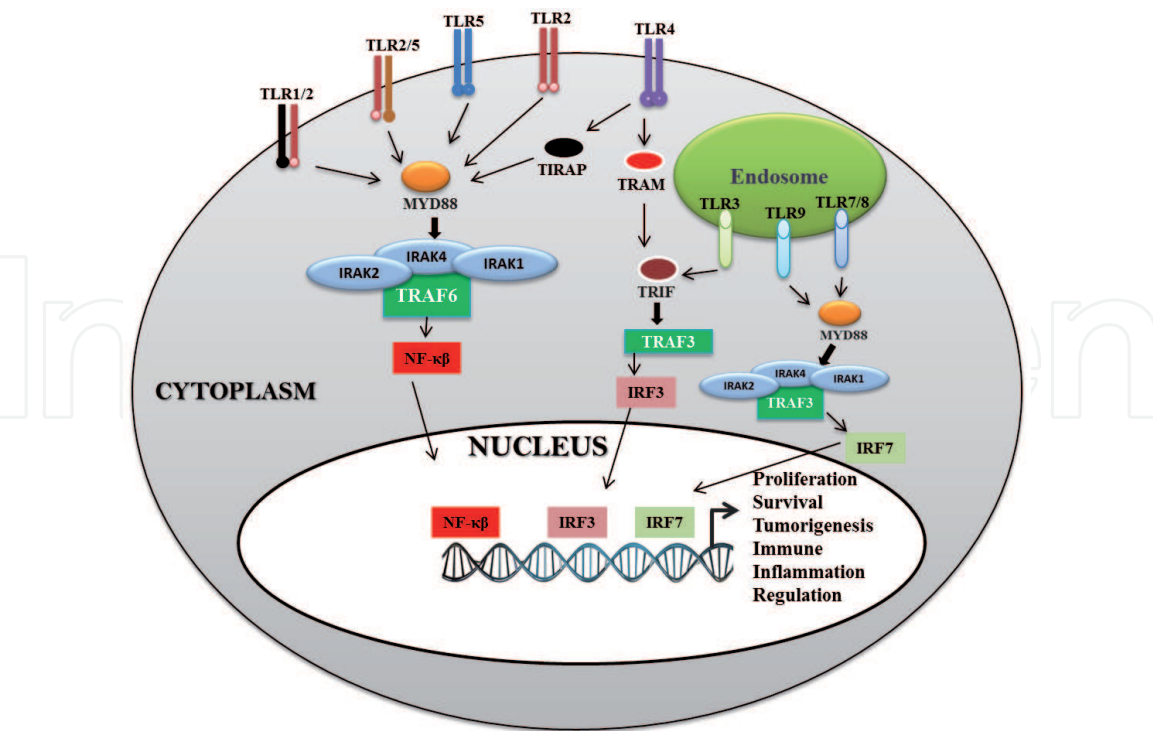


Figure 1.
Toll-like receptors (TLRs) signaling pathway: Toll-like receptors (TLRs) recognize different ligands and triggered innate immune responses. The activation of the TLR signaling pathway originates from the cytoplasmic TIR domain that associates with an adaptor, MYD88. IRAK is activated by phosphorylation and associates with TRAF6, leading to activation NF- κ B. Activation of MYD88- independent pathways occurs via TRIF and TRAF activates interferon--regulatory factor (IRF). Then they promote the transcription of inflammation mediators: Cytokines, chemokines and interferons.

Expression of cytokines also leads to maturation of dendritic cells and activation of B-cells and T-cells, which underlies the involvement of TLR in adaptive immunity [23]. TLR2 and TLR4 upon binding with their respective ligands form dimeric complexes, followed by recruitment of 5 specific adaptors, including 1) MYD88, 2) TIR domain containing adaptor protein (TIRAP)/MYD88 adaptor like (Mal), 3) TRIF, 4) TRIF-related adaptor molecule (TRAM), and 5) sterile α and armadillo motif-containing protein (SARM) [19, 33]. This response elicits the downstream responses like proliferation, invasion, inflammation and tumorigenesis etc. The schematic representation of the role of various TLRs signaling pathways is shown in **Figure 1**. This alternative/non-canonical pathway culminates in the activation of TRAF3 and interferon regulatory factor 3 (IRF3), which results in the secretion of type I IFNs, which are required for an effective antiviral response [31].

3. TLRs biology in the pathogenesis of cancer

In the host cell, TLRs are expressed either on cell membrane or in intracellular compartments (i.e. endosomes) [10]. TLRs belong to a family of pattern recognition receptors (PRRs) that are best-known for their role in host defense mechanism against a number of pathogens. Infection with potential microbial pathogens (bacteria, viruses, protozoa, and fungi) provokes innate and adaptive immune system [26]. In vertebrates, interactions between innate and adaptive immunity leads to highly efficient recognition and clearance of pathogens. Innate immune response elicits nonspecific activation of immune cells (neutrophils, monocytes, macrophages, dendritic cells (DCs), natural killer (NK) cells) and complements system [33, 34]. Inflammation is the immune system's response to protect our body against any harmful stimuli like pathogens, cell damage and harmful/toxic compound. However, uncontrolled acute inflammation may become chronic; contributing to a variety of diseases including cancer [19]. In 1858, Rudolf Virchow noticed that the site of chronic inflammation is highly susceptible to cancer development [35]. He also hypothesized that chronic inflammation could promote the proliferation of cells and thus, the development of cancer. An association between the inflammation and development of cancer has long been appreciated [33]. In 2000, Hanahan and Weinberg proposed a model to define six hallmarks of cancer progression [36]. However, emerging evidence also reiterates the role of inflammation in cancer development. Various studies have shown a close link between chronic inflammation and cancer, such as long standing *H.pylori* infection and gastric cancer [37], chronic pancreatitis and pancreatic cancer, chronic bronchitis and lung cancer, human papillomavirus (HPVs) infection mediated cervical cancer [38], and chronic cholecystitis with gall bladder cancer [33]. Besides inflammatory response, TLR signaling has been shown to regulate apoptosis through the expression of anti-apoptotic proteins or inhibitors of apoptosis [39]. TLRs regulate variety of cellular responses which include the anti-apoptotic effect of NF- κ B, a transcription factor commonly engaged in inflammatory conditions [12, 14]. Although this response can be initiated by several types of PRRs, and TLRs are the best-characterized key players. TLRs also regulate cell proliferation, apoptosis, invasion, and survival by recruiting more immune cells to enhance inflammation in the tumor microenvironment [40]. These tumor cells further release proangiogenic factors and growth factors, which enhance their resistance to cytotoxic lymphocyte attack, thereby leading to immune evasion. As mentioned earlier, TLRs function as double-edged swords, with both pro- and antitumor consequences. However, up-regulation of TLRs in tumor cells may directly or indirectly contribute to carcinogenesis in different organs. Engagement of TLRs on the surface of tumor cells with their ligands can activate subsequent

signaling cascades involving cytokine and chemokine production. Subsequently, these factors can promote tumor invasion, tumor cell survival (apoptosis resistance), chemo-resistance, tumor progression and metastasis.

4. Molecular mechanism of TLRs in cancer progression

Overall, as discussed earlier, the activation of TLRs can promote as well as inhibit tumor growth and cancer progression, but the actual underlying molecular mechanism still remains elusive. TLRs are also involved in controlling many important cellular processes like cell proliferation, survival, apoptosis, cell migration, metastasis and angiogenesis [16]. TLR signaling has been implicated in various autoimmune, chronic inflammation and inflammatory diseases. This situation creates a microenvironment that is rich in growth and survival factors, which leads to the development of various types of cancer [41]. High TLR expression has been reported in several cancer types including cancerous cell lines. It was known that TLR4 and TLR5 are over expressed in gastric epithelium infected with *H. pylori* as well as in precursor lesions [37, 42]. It is considered that TLRs enable cells to interact with *H. pylori* which can induce the expression of tumorigenic factors and may promote cancer development. TLR over-expression has also been found in colon cancer, hepatocellular carcinoma, ovarian and cervical cancers, breast and prostate cancers, lung cancer, melanoma and neuroblastoma [43]. TLR expression in cancer cells has been linked with cancer progression, evasion of immune surveillance, apoptosis and survival. Recent studies have shown high expression of TLR4 in lung cancer cells, which is linked with expression of immunosuppressive cytokines (TGF β), angiogenic factors VEGF and IL-8, and increased resistance to apoptosis [16, 42]. Cell proliferation and production of pro-inflammatory cytokines IL-6 and IL-8 can be significantly decreased by silencing of TLR4 expression in breast cancer cell line (MDA-MB-231) [44]. Other studies in ovarian cancer and cell lines has shown that TLR4 and NF- κ B activation by LPS and paclitaxel respectively promotes production of IL-8, IL-6, VEGF and MCP-1 while TLR4 silencing lead to loss of resistance to Paclitaxel [45]. TLR2 mRNA expression was significantly higher in sporadic colorectal cancer cells than in noncancerous cells [45]. On the basis of above mentioned facts, we can deduce that various TLRs might trigger different signaling pathways in cancer initiation and progression [46].

A recent report found that activation of TLRs may induce cancerous cells to secrete a number of soluble factors, which play distinct roles in cancer development. The role of TLRs in cancer progression needs to be further investigated, and in depth precise underlying mechanism must be elucidated for further development of TLR agonists as therapeutic agents.

5. TLRs modulation in cancer treatments

TLR agonists play an important role in activation of immune system, both innate and adaptive. In *in vivo* models, TLR antagonism have been shown to reduce tumor growth in treatment group, receiving combination of therapeutic agents, such chemotherapy drugs, monoclonal antibodies (mAb), subunit or DNA vaccines [47, 48]. The selection of TLR agonists has been premised on their ability to activate antigen-presenting cells (APCs), particularly DCs. The involvement of specific TLRs on cancer cells may impact tumor growth by various mechanisms, such as inducing apoptosis and potentiating the effects of chemotherapy [45]. Inhibition of TLRs can be achieved either by 1) Preventing the interaction between TLR

and respective binding partner and 2) By blocking the interaction between TLRs and respective adaptor protein. TLR inhibitors can be broadly classified into (i) Small molecule inhibitors, (ii) Antibodies, (iii) Oligonucleotides, and (iv) Lipid-A analogs. The following section illustrates the anticancer effects of inhibiting TLR signaling pathways on tumor growth and developments.

Small molecule inhibitors (SMI): These are synthetic or naturally derived small molecules with the ability to cross plasma membranes due to their small size and amphipathic nature. Interestingly, one of the most commonly used anti-malarial drug chloroquine has been shown to possess inhibitory effects against endosomal TLR7/8/9 [49]. Inhibition of TLR7 and 9 by chloroquine inhibits the growth of hepatocellular carcinoma in both cellular *in vitro* models and mouse xenograft models via down regulation of p-Akt [50]. However due to their non specific mode of actions continuous efforts have been made to develop more efficient and specific derivatives of chloroquine as anti-cancer agents by targeting TLRs. One such derivative, CpG-52,363 has immunosuppressor functions but its role in cancer therapeutics is yet to be discovered [51]. SM934, a derivative of another anti-malarial drug artemisinin can inhibit the proliferation and metastasis in breast cancer probably via inhibition of TLR signaling [52]. TAK-242 specifically inhibits TLR-4 by binding to cysteine 747 in the intracellular domain and consequently suppresses the progression of breast cancer [53]. Therapeutic role of SM934, another artemisinin derivative has been well documented in inflammatory disease [54], but its role in cancer prevention is still need to be explored.

Antibodies: Various antibodies with therapeutic potential have been raised against TLRs to treat a wide spectrum of inflammatory diseases and cancer. Therapeutic role of OPN-305, the first fully humanized IgG against TLR2, against Myelodysplastic Syndromes (MDS) has been reported in different clinical trials [55]. Several antibodies have been developed beside OPN-305, like NI-0101 and T2.5, but their role in cancer has not been determined yet [56].

Oligonucleotides: Specific nucleotide sequences are known to inhibit the function of endosomal TLRs by blocking their binding with respective ligands. These includes immunoregulatory DNA sequence (IRS) 661 (TLR7 specific), IRS-869 (TLR9 specific) and IRS-954 (both TLR7 and 9 specific). Recent report suggest that TLR antagonism using immune modulator oligonucleotide-3100 (IMO-3100) can serve as a potential therapeutic for the management of pancreatic cancer associated cachexia [57].

Lipid A analogs: Eritoran, a syhnthetic analogue of lipid A from *Rhodobacter sphaeroides*, is known to inhibit TLR4 by binding to MD2 pocket and thereby preventing the interaction between TLR4 and lipid A. Bacterial LPS induced colon cancer can be prevented by the administration of Eritoran by mechanism involving inhibition TLR4 and induction of CD14/Src/PKC ζ -mediated apoptosis [58].

It is important to mention here that TLR acts as double edged sword and its agonism can also prevents the progression of cancer by activating the immune response against cancer cells. The following section describes TLR agonists which had shown the potential to prevent cancer progression.

Calmette–Guerin strain (BCG) a live-attenuated *Mycobacterium bovis* can activate TLR2, TLR4, and TLR9. The activation of TLR in urothelial cell carcinomas with BCG induced cell death and decreased proliferation as well as metastasis. The anti-cancer effects of BCG have been associated with increased production of cytotoxic NO in cell lines, as well as in patients [46]. These studies also emphasize the development of vaccination strategies that incorporate TLR ligands to stimulate immune responses and make cancerous cells specific targets for immune system

mediated death. In human colon cancer cells, TLR3 activation with Polyriboinosinic-polyribocytidylic acid (Poly I:C) can induce apoptosis alone or when used in synergy with 5-fluorouracil or IFN- α [16]. Poly I:C is a synthetic analogue of viral dsRNA. The expression of TLR5 on cancer cells has been shown to revoke cell growth in certain types of cancer [16]. For instance, in breast cancer, when TLR5 is over-expression with flagellin inhibits tumor cell proliferation and downregulates expression of cyclin B1, cyclin D1 and cyclin E2 in a murine model [59].

Irradiation along with activation of TLR9 signaling pathway in human glioma cell line can decrease cell proliferation by arresting cell-cycle, which is mediated by NF- κ B and nitric oxide (NO) [60]. This therapeutic effect could be used to sensitize the cancerous cells to the toxic effects of radiation treatment [61]. Also, CpG-island mediated activation of TLR9 in neuroblastoma cell has been revealed to decrease cell proliferation and increase caspase-dependent apoptosis and leads to an increased survival in tumor-bearing mice. Several TLR agonists have been approved by the food and drug administration (FDA) for use in the treatment of cancer patients like BCG (which activate TLR2, TLR3, TLR4, and TLR9), MPL (TLR4 agonist) and imiquimod (TLR7 agonist) [62]. TLR agonists should be used in combination with other agents to synergistically increase their immune stimulatory response. An important TLR modulators are summarized in **Table 1** which having anticancer activity.

Future direction of TLRs based treatment of cancer:

In this book chapter, we summarized the role of TLRs signaling in inflammation, cell proliferation, apoptosis and chemo-resistance, which are the major attributes of cancerous cells. Beside these, several TLRs agonists and antagonists have been developed and/or are in clinical trials as cancer therapeutics. TLRs play a critical role in imparting immunity against tumor, and their antitumor effects are noticeable as depicted from previous studies. It is quite interesting to note that activation of same TLR in one tumor type might induce cell death, and in a different tumor could exert pro-tumor effects. Using TLR agonists or antagonist as cancer therapeutics must be decided on the basis of TLR expression profile of tumor cells and resulting response within a specific cancer type [19]. The prospective approach for future cancer treatment will be the combination of specific TLR agonists or antagonists with traditional cancer treatments to improve treatment outcome. The role of TLRs in both promoting and inhibiting tumor growth and metastasis has been confirmed in various studies. However, the specific mechanism of action is still unclear as cancer is a multifactorial\ disease, and the research of TLRs on tumor immunity is still in the nascent phase. Further in depth studies will help us to develop better

Name	Targets	Antagonist/ agonist	Ref.
Chloroquine	TLR 7 and 9	Antagonist	[50]
TAK-242	TLR 4	Antagonist	[53]
IMO-3100	TLR 7, 8 and 9	Antagonist	[57]
Eritoran	TLR 4	Antagonist	[58]
Polyriboinosinic-polyribocytidylic acid (Poly I:C)	TLR 3	Agonist	[16]
Calmette–Guerin strain (BCG)	TLR 2, 4 and 9	Agonist	[46]

Table 1.
Different TLR modulators having anticancer activity.

understanding of TLRs role in tumorigenesis, tumor immunity, and tumor metastasis which in turn can provide new strategies and prospects for more effective cancer management. We anticipate that future studies on the role of TLRs in cancer progression and development will provide us a better insight into the mechanisms underplaying. Therefore, understanding the roles of TLRs in tumor biology may pave the way for the discovery of novel therapeutic targets in cancer therapy.

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